Microbiological Safety of Blue and Cheddar Cheeses Containing Naturally Modified Milk Fat

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ABSTRACT

Milk containing naturally modified fat was obtained by feeding lactating dairy cows a Control diet and two experimental diets containing either extruded soybeans or sunflower seeds. Milk from cows fed the experimental diets contained higher levels of both long chain (C₁₈:₀) and unsaturated fatty acids than the milk from cows fed the Control diet. Each milk was pasteurized, standardized to 3.6% milk fat, and inoculated with Listeria monocytogenes (strains Scott A and V7), Salmonella typhimurium and Salmonella senftenberg, before manufacturing into Blue or stirred-curd Cheddar cheeses. Populations of L. monocytogenes and Salmonella spp. were monitored during manufacture and aging using Oxford and Xylose Lysine Desoxycholate agars, respectively. During the manufacture of Blue and Cheddar cheese, and during the aging of Blue cheese, behavior of Salmonella spp. and L. monocytogenes in the experimental cheese was similar to the Control cheese. During aging of Cheddar cheese, the rate and extent of decline of Salmonella spp. and L. monocytogenes varied among the cheeses. Declines correlated with the accumulation of specific fatty acids, namely C₁₇:₀, C₁₈:₀, C₁₈:₁ and C₁₈:₂. These fatty acids were also found to be inhibitory to S. typhimurium and L. monocytogenes when incorporated into tryptic soy agar plates at 37°C. Therefore, the natural fat modification of Blue and Cheddar cheeses enhanced the safety of these cheeses.

Key words: Cheese, fat, Listeria, Salmonella

Recent consumer trends for foods low in total fat, saturated fat and cholesterol have caused the food and dairy industries to explore and produce new food products to meet consumer demands. The dairy industry has responded by developing new low-fat and non-fat yogurt, cheese and frozen desserts. It has also developed lower cholesterol-containing milk and butter blends. Consumer preferences for not only lower amounts of fat, but also higher proportions of unsaturated instead of saturated fat in the diet is also evident. One problem the dairy industry faces when developing new types of lower fat and lower cholesterol-containing products is meeting the standards of identity for each particular product. Cheeses manufactured from milk containing polyunsaturated oil blends (filled milk) do not meet the standards of identity (1) for natural cheese. Such imitation cheeses also have inferior sensory qualities (19,20,21).

Thus, one approach to increase the desirability of cheese is to increase the proportion of its unsaturated fat content by supplementing portions of the diets of lactating dairy cows with sunflower seeds (3,6,14) or soybeans (14). Cheddar cheese has been manufactured using milk with this naturally modified fat and it was found to have acceptable flavor as well as good manufacturing and storage characteristics (14).

With respect to microbial safety of dairy products, pasteurization is commonly used to eliminate pathogens from raw milk before it is further processed into dry milk, ice cream and other dairy products. The current basis for the microbial safety of cheese is to use pasteurized milk or age the cheese, if it is made from raw or unpasteurized milk, for 60 days (at ≥3.5°C). During aging, pathogens are expected to die because of the effects of low pH and organic acids. However, the effectiveness of this aging process in destroying pathogens is questionable because experimental as well as commercial cheeses were found to contain food borne pathogens even after 60 days of aging (4,9,10,18,22).

During aging, pathogens are exposed to lactic acid (pH 4.5 to 5.5) and other inhibitory compounds (acetic acid, hydrogen peroxide, etc.) produced by lactic acid bacteria (starter cultures). Free fatty acids released during aging also contribute to the destruction of food borne pathogens in aged cheese. Unsaturated fatty acids have been shown to have detrimental effects on some food borne pathogens in various microbiological media and in food systems (8,11,12,24). If cheeses were to contain higher proportions of unsaturated fats, the specific fatty acids released during aging may have a greater detrimental effect on pathogens than the regular fatty acids found in normal cheeses.

In young Cheddar and Blue cheeses of pH 4.5 to 5.3, gram-negative organisms are primarily destroyed by prolonged exposure to acidity and short chain acids, whereas gram-positive bacteria which survive lengthy exposure to...
low pH, may be destroyed by longer chain fatty acids, or mono- and diglycerides released during aging.

The purpose of this research was to investigate the behavior of _L. monocytogenes_ and _Salmonella_ spp. during manufacture and aging of Cheddar and Blue cheese made from milk having a naturally altered fat composition.

**MATERIALS AND METHODS**

**Bacterial cultures**

_Listeria monocytogenes_ Scott A, _L. monocytogenes_ V7, _S. typhimurium_ Hill Farm and _S. senftenberg_ from our culture collection were used as inocula. These cultures were grown in 10 ml of tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI) plus 0.6% yeast extract (Difco) for 18 to 24 h at 30°C followed by storage at 4°C. Working cultures were grown in sterile 11% (wt/vol) reconstituted skim milk (RSM, Land O’ Lakes, Arden Hills, MN) at 37°C for 18 to 24 h followed by a second identical transfer. The cultures were diluted with 0.1% (wt/vol) peptone (Difco) water before adding to pasteurized cheese milk.

**Milk source and handling**

Two lots of milk were obtained from South Dakota State University in which cows were fed Control and modified rations containing soybeans and sunflower seeds (14). Each of the milks was collected separately, standardized to 3.6% milk fat, batch pasteurized, cooled to 4°C and poured into 5-gallon plastic bags. These bags were held 18 h at 2°C before placing into coolers with ice and transported to the University of Minnesota. The milk was stored another 16 h at 2°C prior to cheese manufacture.

**Cheddar cheese manufacture**

Six liters of each milk (Control, Soybean, Sunflower) were each placed in one of three small (6.5 L) rectangular stainless steel vats. All three vats were placed in a water bath equipped with automatic stirrers. Each milk was warmed to 31°C and the following were added: 0.022% (wt/vol) of commercial pelletized starter culture (Chr. Hansen’s, Milwaukee, WI. Phage Control Culture R604), 0.02% (vol/vol) of single strength CaCl₂ (Chr. Hansen’s), _10³ CFU/ml of _S. typhimurium_ HF and _S. senftenberg_, each grown in RSM.

The procedure for the manufacture of stirred-curd Cheddar cheese was a modification of Kosikowski’s method (13). The differences between the procedure used for this research and that of Kosikowski’s procedure were: (a) rennet was added 1 h after the addition of the starter culture rather than 30 min; and (b) the curd was salted at a titratable acidity (TA) of 0.40% rather than 0.25 to 0.35%.

After salting the curd, it was dispensed into ~0.5 kg gouda cheese hoops and pressed at 14 to 18 kg of pressure for 16 h. After pressing, the cheese wheels (~0.6 kg each) were vacuum-packaged and aged at 7°C.

**Blue cheese manufacture**

Twenty-seven liters of each milk were poured into 45 L capacity stainless steel tubs. These were placed in a large water bath. The milk was warmed to 31°C and the following were added: 0.044% (wt/wt) pelletized frozen starter (Chr. Hansen’s Phage Control Culture R604), 0.02% (vol/wt) single strength CaCl₂ (Chr. Hansen’s), 0.0022% _Penicillium roqueforti_ spore powder (DFL Midwest, Waukesha, WI), 10⁻⁴ CFU/ml _L. monocytogenes_ (Scott A and V7) and 10⁶ to 10⁷ CFU/ml of _S. typhimurium_ HF and _S. senftenberg_, each grown in RSM.

The Minnesota method using unhomogenized milk for Blue cheese manufacture, as described by Morris (17) was followed for this research except calf lipase powder (Capalase K, Chr. Hansen’s) was added (0.0031% wt/wt) to the milk immediately before cheese manufacture.

After manufacture, the cheeses were removed and placed into a glass chamber (31 cm x 32 cm x 76 cm, -98% RH using saturated Na₂SO₄ solution) and kept at 13°C for 30 to 60 days to allow proper mold growth before storing at 4°C.

**Sampling procedures for Cheddar and Blue cheeses**

Duplicate samples of milk, whey and/or curd were collected for microbial analysis at the following stages of Cheddar cheese manufacturing: initial inoculation, after cutting and at hooping. Twenty-four-hour and 1-, 2-, 3- and 4-month-old Cheddar cheese samples were collected and analyzed for microbial populations and pH. Samples at 24 h and 2 months were analyzed for free fatty acids. Samples for fatty acid determinations were stored at -20°C until analyzed.

For Blue cheese, samples were collected in duplicate and plated for microbial numbers at the initial inoculation, partial draining, hooping and at the final drain (4-h) stages. Samples of 10-day and 1-, 2-, 3- and 4-month-old cheese were examined for microbial numbers and pH.

**Enumeration of _L. monocytogenes_**

Duplicate samples (0.1 to 0.25 ml) of milk or whey were surface plated onto 2 to 4 Oxford agar (Unipath-Oxoid, Inc., Ogdenburg, NY) plates (15 mm x 100 mm) and incubated at 37°C for 48 h. Curd or cheese samples (10 g) were stomached (Tekmar Co., Cincinnati, OH) in warm, distilled water (35 to 40°C, 90 g) containing 2% (wt/vol) sodium citrate, for 3 min before surface plating 0.1 ml of the cheese slurry or dilution onto Oxford agar plates. Dilutions were prepared with sterile 0.1% peptone (Difco) water.

During aging when the numbers were expected to decline to ≤10 CFU/g, the Most Probable Number (MPN) technique was used in which 3 to 10 g, 3 to 1 g, and 3 to 0.1 g samples were examined using the Food and Drug Administration method for the enrichment of _Listeria_ spp. in cheese (15). Cheese samples were added to warm (35°C) U.S. Food and Drug Administration (FDA) _Listeria_ enrichment broth at a 10% level, stomached for 3-min and the pH was adjusted to 7.3 ± 0.2 with sterile 5 N sodium hydroxide. Following pH adjustment, the enrichment broths were incubated at 30°C and subsequently streaked onto Oxford agar plates at 24 and 48 h.

**Enumeration of _Salmonella_ spp**

Direct plating for _Salmonella_ spp. was performed as described for _L. monocytogenes_ except that xylose lysine desoxycholate (XLD, Difco) agar plates were used.

During aging when numbers were expected to decline to ≤10 CFU/g, the MPN procedure was used as for _Listeria_ spp. except that the cheese samples were added to warm (35°C) lactose broth (Difco) and incubated for 24 h at 37°C. Following this pre-enrichment, 1 ml of lactose broth was added to 9 ml of tetraphionate broth (Oxoid) and another milliliter was transferred to 9 ml of selenite cystine (Oxoid) broth. The selective enrichment broth tubes were then incubated for 24 h at 37°C before streaking onto XLD (Difco) agar plates which were also incubated at 37°C for 24 h.

**Free fatty acid analysis of cheese samples**

The free fatty acid profiles for 24-h and 2-month-old samples of Cheddar cheese were determined. The free fatty acids (FFA) from these samples were extracted according to the method of Deeth et al. (5), except all ingredients were increased 5-fold. Pentanoic acid was used as the basis to quantify short and medium chain (C₄ to C₁₆) fatty acids, while decaheptanoic acid was used to
quantify long chain (C₁₄ thru C₁₈) fatty acids. The amount of internal standard (100, 200, 400, 500, 1,000, 2,500 or 3,000 µg) added to each sample depended upon the age of the cheese being examined. Two microliters of the supernatant which contained the FFAs were injected into an HP 5890A gas chromatograph (Hewlett Packard Co., Palo Alto, CA) fitted with a ChemStation and flame ionization detector. The column used was a DB-5 (30 m × 0.32 mm i.d. × 0.1 µm film thickness, J & W Scientific, Folsom, CA). Each sample was injected twice, once to quantify short and medium chain fatty acids (C₁₄ thru C₁₈) and again to quantify long chain fatty acids (C₁₈ thru C₂₀). The GC injector port and detector temperatures were set at 220°C and 250°C, respectively. The temperature program used to quantify C₁₈ through C₂₀ fatty acids was set at an initial time of 0 min, an initial temperature of 200°C, and a heating rate of 3°C/min. When 250°C was reached, this final temperature was held for 3 min to complete the run. The temperature program to quantify C₁₈ thru C₁₈:2 fatty acids consisted of an initial time of 0 min and an initial temperature of 200°C, and a heating rate of 3°C/min. When 250°C was reached, it was held for 3 min. The split was set at 1 to 50 (sample:gases) for both runs. Relative response factors (RRF) were calculated and retention times determined by dissolving ~0.01 g of each fatty acid in diisopropyl ether and passing them through the GC. The fatty acids were then quantified by relating the corrected peak areas of each fatty acid to the peak area of the C₁₆ or C₁₈ internal standard.

Titratable acidity, pH, fat, NaCl and moisture analyses

Titratable acidity of milk and whey was measured at the time of bacteriological enumeration as described by Case et al. (2). The pH of milk (~2 ml) and whey (~2 ml) was measured using a combination glass pH electrode (Radiometer America, Inc., Westlake, OH). The pH of the cheese and curd was measured by placing ~1 g of sample and ~1 ml of deionized water into a 6 oz. Whirl-pak™ (Nasco, Ft. Atkinson, WI) bag and stomaching (Tekmar) until a slurry was formed. The pH of the slurry was then measured using a combination glass pH electrode (Radiometer America).

Fat, NaCl and moisture analyses for all cheese samples were carried out in duplicate as described by Case et al. (2). The Mojonnier and Mohr methods were used for fat and salt analyses, respectively.

Preparation of plating media containing fatty acids

filter sterilized C₁₂, C₁₄, C₁₆, C₁₈, C₂₀ and C₂₂ (≥99% purity, Nu-check Prep, Elysian, MN) each were weighed into test tubes (18 × 150 mm) and dissolved in 95% ethanol. Appropriate portions of the dissolved fatty acid solutions were added to 1 L flasks of tempered (50°C) trypsic soy agar (TSA; Difco) to obtain 250, 500 and 1,000 µg of fatty acid per ml of TSA. The contents of each flask was thoroughly mixed before pouring ~30 ml of TSA into each petri plate (15 × 100 mm) and allowing to solidify at room temperature.

 Cultures of L. monocytogenes and S. typhimurium were incubated 24 h at 37°C in TSB and diluted in 0.1% peptone water to obtain ≤250 CFUs when 0.1 ml of the suspension was surface plated. After plating, the plates were incubated at 37°C for 48 h before counting. Plates were re-examined after being held for 7 days at room temperature to be sure that slower forming colonies, if any, were not omitted.

RESULTS AND DISCUSSION

Composition of modified fat Blue and Cheddar cheese

The percentages of fat in dry matter (FDM), salt, moisture and salt in moisture (S/M) for the cheeses are shown in Tables 1 and 2. The United States standards of identity for Blue cheese requires a minimum of 50% milk fat by weight in the dry matter and a maximum moisture content of 46% by weight (1). Only one cheese (Soybean in trial 2) did not meet this criterion; the FDM was <47.2%. The cheeses varied in salt content and salt-in-moisture from 4.2 to 5.5% and 9.1 to 13.5%, respectively. The pH of the cheeses after hooping ranged from 4.8 to 5.0, which is typical for Blue cheese.

All six Cheddar cheeses met the U.S. standards of identity, ≤39% moisture and at least 50% FDM (Table 2) (1). Cheddar cheese generally has a pH of 5.1±0.2 and, as shown in Table 3, all of these cheeses fell within this range. Further, the pH of the cheese did not seem to vary with the type of fat or degree of unsaturation.

Behavior of Salmonella spp. during the manufacture and ripening of Blue cheese

Figure 1 shows the number of Salmonella spp. in Blue cheese during manufacture and ripening. The initial inocu-
TABLE 3. Average* pH values of Cheddar cheese during ripening.

<table>
<thead>
<tr>
<th>Type</th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.02 (.04)b</td>
<td>5.20 (.07)</td>
</tr>
<tr>
<td>Soybean</td>
<td>5.02 (.04)</td>
<td>5.16 (.05)</td>
</tr>
<tr>
<td>Sunflower</td>
<td>5.12 (.04)</td>
<td>5.22 (.08)</td>
</tr>
</tbody>
</table>

* Average of duplicate samples taken at 30, 60, 90 and 120 days.

b Standard deviation.

Figure 1. Behavior of Salmonella spp. during the manufacture and ripening of Blue cheese.

Figure 2. Profiles of pH during ripening of Blue cheese.

Others (16). This pattern of decline of Salmonella spp. in the modified fat cheeses was similar to that seen in the Control cheeses.

Behavior of L. monocytogenes during the manufacture and ripening of modified fat Blue cheese

The behavior of L. monocytogenes is shown in Fig. 3. The initial numbers were 3.4 log CFU/ml of milk in both trials. The numbers of L. monocytogenes after hoopage reached a maximum of 4.5 log CFU/g in all cheeses. This pattern of similar numbers in all three cheeses was similar to that of the Salmonella spp. seen earlier and to that reported for Listeria by others (18).

During aging, L. monocytogenes followed the same pattern of decline in the modified fat cheeses to that seen in the Control cheeses. Despite an increase of pH in all cheeses from 4.9 to 6.8 between 60 and 120 days, L. monocytogenes did not increase in numbers. Listeria monocytogenes was shown to increase significantly in mold-ripened soft cheeses such as Camembert and Brie, when pH increases (23). In our Blue cheeses, this increase was not seen and this might be due to the effect of higher amounts of free fatty acids (FFAs, 18 to 25% [7]) and/or higher salt concentrations (S/M) than in Camembert or Brie cheeses (9% versus 5%).
Behavior of Salmonella spp. in modified fat stirred-curd Cheddar cheese.

The behavior of Salmonella spp. in modified fat Cheddar cheese is shown in Fig. 4. Initial numbers of Salmonella spp. ranged from 2.5 to 3.1 log CFU/ml of milk. At draining, numbers increased in the curd and, after pressing, they ranged from log 4.1 to 4.9 log CFU/g. Numbers in trials 1 and 2 were similar in each cheese after pressing.

Although, Salmonella spp. decreased in all cheeses during ripening, the rate of decline varied. In trial 1, faster declines were observed in the Control and Sunflower cheeses than in the Soybean cheese. In trial 2, Salmonella spp. declined faster in the Soybean cheese than in the other two cheeses. Cheeses which had faster declines also contained higher overall levels of FFAs and also specific FFAs at 60 days (Table 4). In trial 1, it was the Control (0.22% (wt/wt) FFA/g fat) and Sunflower (0.96%) cheeses while in trial 2, it was the Soybean (0.42%) cheese.

In trial 1, the Sunflower cheese had significantly higher amounts of medium chain acids (>2.5 fold), and C\textsubscript{18:1} and C\textsubscript{18:2} (>11.5 fold) as compared to the Control and Soybean cheeses. In trial 2, it was the Soybean cheese that contained significantly higher amounts of medium chain acids (>1.6 fold), and C\textsubscript{18:1} and C\textsubscript{18:2} (>5.8 fold). These differences in the overall FFAs and concentrations of specific FFAs between the two trials might be due to a difference in the natural lipases and lipolytic enzyme activity. The types of FFAs in combination with the total amount may have been responsible for the faster declines.

Table 4. Accumulation of free fatty acids in Cheddar cheeses.

<table>
<thead>
<tr>
<th>Days of Aging (µg/g cheese)</th>
<th>0</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Soy</td>
<td>Sun</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Trial 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short\textsuperscript{a}</td>
<td>43</td>
<td>38</td>
</tr>
<tr>
<td>Medium\textsuperscript{b}</td>
<td>260</td>
<td>270</td>
</tr>
<tr>
<td>Long\textsuperscript{c}</td>
<td>61</td>
<td>113</td>
</tr>
<tr>
<td>C\textsubscript{12}</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>C\textsubscript{14}</td>
<td>56</td>
<td>54</td>
</tr>
<tr>
<td>C\textsubscript{16:1} &amp; C\textsubscript{18:2}</td>
<td>39</td>
<td>74</td>
</tr>
<tr>
<td>% F.A. released</td>
<td>.11</td>
<td>.12</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Trial 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short\textsuperscript{a}</td>
<td>54</td>
<td>14</td>
</tr>
<tr>
<td>Medium\textsuperscript{b}</td>
<td>288</td>
<td>93</td>
</tr>
<tr>
<td>Long\textsuperscript{c}</td>
<td>57</td>
<td>52</td>
</tr>
<tr>
<td>C\textsubscript{12}</td>
<td>23</td>
<td>7</td>
</tr>
<tr>
<td>C\textsubscript{14}</td>
<td>65</td>
<td>22</td>
</tr>
<tr>
<td>C\textsubscript{16:1} &amp; C\textsubscript{18:2}</td>
<td>41</td>
<td>37</td>
</tr>
<tr>
<td>% F.A. released</td>
<td>.11</td>
<td>.05</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Short (C\textsubscript{6}-C\textsubscript{8}).
\textsuperscript{b} Medium (C\textsubscript{10}-C\textsubscript{16}).
\textsuperscript{c} Long (C\textsubscript{18}-C\textsubscript{18:2}).
When FFAs were incorporated into TSA at pH 7.0, oleic acid resulted in 64 to 70% inhibition of S. typhimurium whether present at 250, 500 or 1,000 μg FA/ml (Fig. 5). The inhibitory effect of lauric and myristic acids was concentration dependent; 24%, 49% and 94% inhibition with lauric acid, and 6%, 16% and 57% inhibition with myristic acid at 250, 500 and 1,000 μg FA/ml TSA, respectively. Even though the actual concentrations of these FFAs were smaller in the cheeses at 60 days than was required to cause 50 to 95% inhibition in the agar plate system, the combined effects of lower pH (5.2 versus 7.0), lower temperature (7°C versus 37°C) and various FFAs may have caused the faster decline of salmonellae.

Behavior of L. monocytogenes in modified fat stirred-curd Cheddar cheese

The behavior of L. monocytogenes during the manufacture and aging of modified fat Cheddar cheese is shown in Fig. 6. Each of the milks were inoculated with 3.2 log CFU/ml. They were further concentrated and/or grew in the curd and were at levels of 4.2 to 4.7 log CFU/g after pressing in all cheeses. The behavior of L. monocytogenes in the Control cheeses during manufacture was similar to that found by others (22).

During aging, L. monocytogenes declined at different rates in these Cheddar cheeses. A faster decline was seen in the Sunflower cheese than in the Control and Soybean cheeses in trial 1. The opposite was seen in trial 2, where the decline of L. monocytogenes was faster in the Soybean cheese than in the Control or Sunflower cheeses. Also in trial 2, there was a faster decline of L. monocytogenes in the Sunflower than in the Control cheese. Since all the cheeses were similar with respect to pH, moisture and salt, the differences of decline between the cheeses in both trials may relate to the differences in the amounts of specific fatty acids that accumulated during aging.

In both trials, the cheese that contained the highest amount of FFAs had the fastest decline of L. monocytogenes. In trial 1 it was the Sunflower cheese, which contained 0.96% FFAs while the Control and Soybean contained only 0.22 and 0.19%, respectively (Table 4), at 60 days. At 120 days of aging, the organism was no longer detected in the Sunflower cheese. Levels of oleic and linoleic acids were also higher in the Sunflower (>11.5 fold) than the Control and Soybean cheeses. This may have contributed to the faster decline of L. monocytogenes in the Sunflower cheese. Both of these acids as well as lauric and myristic acids were found to have inhibitory effects on L. monocytogenes Scott A in TSA (Fig. 7). Only lauric and linoleic acids caused 100% inhibition, when present at a concentration of 1,000 μg FA/ml TSA. As stated earlier, lower concentrations of these acids in the cheeses may have caused the declines due to the combined effects of pH.
FFAs and temperature.

As with *L. monocytogenes* in the first trial, in trial 2 the fastest decline again was seen in the cheese that contained the highest amount of FFAs. The Soybean cheese had a faster decline of *L. monocytogenes* and contained 0.42% FFAs. The Control and Sunflower cheeses had slower declines and contained 0.17% and 0.13% (Table 4), respectively. Even though the Sunflower cheese in trial 2 contained less FFAs than the Control cheese, *L. monocytogenes* declined at a faster rate and to a greater extent. This might be because of the higher concentration of oleic and linoleic acids in the Sunflower cheese.

In general, the cheeses which contained the highest level of FFAs also had the fastest decline of organisms. Not only the amounts but also the types of FFAs in the modified Cheddar cheeses seem to play an important role in the destruction of *Salmonella* spp. and *L. monocytogenes* during the aging process. Even though only the effects of individual fatty acids were studied in microbial media, the detrimental effects noted in the cheeses were expected to be the result of the combined effects of various FFAs, pH and salt content.

Thus, Blue and Cheddar cheeses made from milk containing naturally modified milk fat are safe. This enhanced safety appeared to be the result of the inhibitory effects of free fatty acids released during aging.

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